

## A method for measuring the ECG and ventilation rate in bats

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**Abstract.** A new method is described for the simultaneous measurement of ventilation rate and the parameters of the electrocardiogram (ECG) for bats. The ECG was measured using mildly adhesive electrode pads that were placed on the forearms of the animal. Ventilation rate was simultaneously measured by recording the bat's thorax movements with a human pulse transducer placed beneath the bat while it was housed in a specialised chamber. These methods reduced the risk of permanent injury to the bat and, unlike other methods used, did not cause any physical damage to the bat's body. The clarity of the signal found using this new method was demonstrated for Western Australian bats at heart rates ranging from  $120 \text{ b min}^{-1}$  up to  $720 \text{ b min}^{-1}$ .

**Key words:** bat, ECG, ventilation, methodology

### Introduction

Measurement of the electrocardiogram (ECG) was first demonstrated in 1909 by Augustus Waller using a dog standing in jars containing salt that were linked to two galvanometers (Levick 1995). The methods used, and our understanding of the information contained in an ECG, have improved considerably since then. The requirements for measurement of the ECG of animals, particularly small mammals, has undergone a dramatic change in recent times as it has become increasingly important to use methods that minimise stress and intrusion.

Most physiological studies measuring the ECG of small mammals have used sub-dermal insertion of thin metal electrodes at various points around the body (e.g. Chatfield & Lyman 1950; Twente & Twente 1978; Milsom *et al.* 1993; Harris & Milsom 1995). While this technique maximises the clarity of the signal, there is a risk to the animal associated with the anaesthesia required and an increased risk of permanent damage due to post-surgery infection. Recently, studies involving one of the smallest mammals (*Suncus etruscus*) have utilised a non-intrusive method of ECG measurement involving an electrode grid which the animal stands on to provide contact between the skin and the leads that record the ECG (Fons *et al.* 1997).

While significant progress has been made in this respect for recording of ECGs in most small mammals, it appears there has been little change in the methods used to measure the same parameters in bats. The only method employed has been one that is similar to that described previously involving sub-dermally inserted electrodes (Twente & Twente 1978; Cook *et al.* 1987). In the case of bats, these electrodes were inserted through the wing membrane in a number of places. Again, while this ensures good quality of the ECG signal, there is a risk of death during the insertion of the electrodes under anaesthesia, and of the possibility of permanent damage to the delicate wing membrane.

I describe here a new non-invasive technique for monitoring the ECG and ventilation rate in bats, and demonstrate its effectiveness over a range of activity levels.

### Materials and Methods

Four different species of bat ranging in size from 5 to 21 g were used to investigate the effectiveness of this technique across a range of size classes. These species included lesser and greater long-eared bats (*Nyctophilus geoffroyi* and *Nyctophilus timorensis*), Gould's long-eared bat (*Nyctophilus gouldi*) and Gould's wattled bat (*Chalinolobus gouldii*). Recordings of the ECG and ventilation rate were made during euthermia and during torpor at an ambient temperature of 15 °C.

For the measurement of the ECG and ventilation rate, bats were placed in a 'holder' consisting of a vertically suspended, 15 cm length of 30 mm dia PVC pipe lined with hessian to provide a surface from which the bats could hang. The lid consisted of a removable, modified cap for the PVC pipe with holes cut in either side to allow the ECG leads (described below) to move freely as the animal moved in the tube. The opposite end had a similar cap with a hole in the centre to allow airflow into the holder. This end of the holder was also lined with hessian to prevent the animal from trying to force its way out of the hole.

The ECG electrodes were placed on the bat to measure a Lead I signal (the hardware and software were only capable of measuring one ECG signal). In humans, a Lead I ECG measures cardiac activity by recording the difference in potential between the left and right arms. Similarly, in the case of the bats, the positive and negative electrodes were placed on the bat's forearms just distal to the elbow. These electrodes, with the leads attached, were placed on the bat while outside the chamber. The bat was then placed near the entrance to the chamber and allowed to crawl in before sealing the end of the chamber with the cap.

While this electrode system is commonly used in humans, the leads and the electrodes themselves required modification for ECG measurement of bats weighing less than 25 g. The leads themselves consisted of a length of insulated electrical wire (0.65 mm dia) with a plug at one end that was insulated and crimped to fit the input sockets of a 3 lead Bio Amp cable (ADI Instruments). The other end of the lead was a modified miniature clip for attachment to electrode pads that adhered to the bat. To fashion a miniature clip, a brass safety pin was cut in half, leaving the spring with two ends measuring approximately 0.5 cm. The tips of the ends were flattened and twisted around each other to form a clip that shut under its own spring tension. The modified clip, except for the tips that were in contact with the electrode pads, was then coated in a clear lacquer to ensure maximum insulation and minimise interference. These leads, and the associated clips, were sufficiently strong to ensure that a continuous circuit was maintained between the electrode pads and the hardware, but were also light enough so that the animals could move with a minimum of hindrance. The electrode pads were strips cut from adhesive Bio Tab (ADI Instruments) silver chloride electrodes that were then clipped to the end of the electrode leads. These electrodes could be placed on the bat without the need for anaesthesia and appeared to cause minimal disturbance to the bat once they were attached. The mildly adhesive nature of the electrodes meant that they could be removed without damaging the delicate wing membrane.

The ventilation detector consisted of a MLT1010 pulse transducer (ADI Instruments) placed beneath the hessian strip in the 'bat holder'. This transducer is typically used to measure heart rates from a finger pulse in humans. For application as a ventilation detector, the only modification that was required was the removal of the strap that usually secured the transducer in place around the finger. The transducer was placed beneath the hessian before the bat entered the chamber. Its sensitivity was such that any movement of the bat's thorax could be monitored with

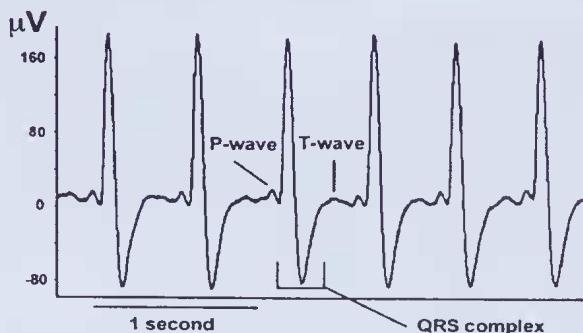


Figure 1. ECG recording from a lesser long-eared bat (*Nyctophilus geoffroyi*) during torpor with a heart rate of approximately  $120 \text{ b min}^{-1}$ . The atrial contraction (P-wave) ventricular depolarisation (QRS complex) and ventricular repolarisation (T wave) can be clearly distinguished.

minimal disruption to its normal mobility. From these thorax movements it was possible to calculate ventilation rate.

The Bio Amp cable was connected to a ML132 Bio Amp (ADInstruments). The amplified ECG signal and pulse transducer signal were interfaced to a MacLab (ADInstruments, v 4/s) and Macintosh personal computer (LC630), allowing the inputs to be recorded using Chart (ADInstruments, v 3.5.6). This system allowed simultaneous recording of ventilation rate and heart rate and had sufficiently high sampling rates (1 to 100 kHz) such that the major components of the ECG could be identified.

#### Results and Discussion

This system was highly effective over a wide range of activity levels for all bat species tested. At low heart rates, it was possible to distinguish the depolarisations of both the atrial (P wave) and ventricular (QRS complex) chambers from the ECG (Fig 1). At higher heart rates, however, this distinction became less clear (Fig 2). This was mostly due to the limitations of the hardware and software rather than a limitation of the technique. The most common sampling rate used was 1 kHz. At high heart rates this sampling rate was not sufficient to distinguish the components of the ECG (Fig 2). At higher sampling rates, however, it was only possible to record one channel at a time, so for the purposes of simultaneously measuring heart rate and ventilation rate the resolution was compromised.

Despite this, even at relatively low sampling rates the system demonstrated a clarity that was equal to, if not better, than that using the method of sub-dermal electrode insertion (see Twente & Twente 1978; Harris & Milsom 1995) and was certainly more effective than the method used on other mammals weighing less than 25 g (see Fons *et al.* 1997). Similarly, the sensitivity of the pulse transducer ensured that ventilation rate could be easily monitored (Fig 3) allowing an immediate comparison to be made between heart rate and ventilation rate.

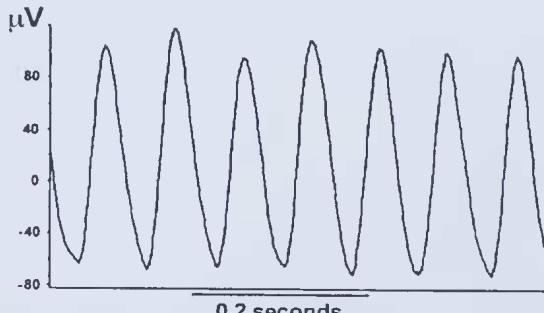


Figure 2. ECG recording from a Gould's long-eared bat (*Nyctophilus gouldi*) with a heart rate of nearly  $720 \text{ b min}^{-1}$ . The only part of the ECG that was recorded at a sampling rate of 1 kHz was the QRS complex.

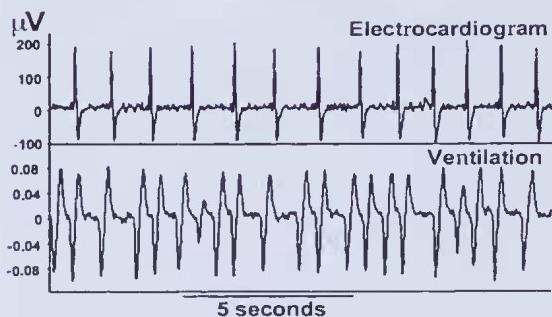


Figure 3. Simultaneous recording of an ECG and ventilation events from a lesser long-eared bat (*Nyctophilus geoffroyi*).

On occasions, due to their design the electrode pads were dislodged as the bat entered the chamber. However, it was essential that the pads were only mildly adhesive so that they could be removed without damaging the delicate wing membrane. This also allowed the bat the ability to free itself from entanglement and was seen as an advantage over the method involving sub-dermal insertion of wire electrodes.

This method of ECG measurement eliminated the risk associated with anaesthesia and did not cause any damage to the bat's body. Given that there was minimal intrusion on the bats, this methodology has significant implications for further research into the cardiorespiratory physiology of bats. This subject is of particular interest given that bats represent an extreme in terms of cardiac neuronal control (O'Shea & Evans 1985; O'Shea 1987; O'Shea 1993) over a range of heart rates from less than  $10 \text{ b min}^{-1}$  during torpor (Twente & Twente 1978) up to  $1000 \text{ b min}^{-1}$  during flight (Thomas & Suthers 1972).

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